

## The infantile-onset form of Pompe disease: an autopsy diagnosis

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### ABSTRACT

Pompe disease (PD) is a rare, inherited autosomal recessive metabolic disorder caused by the deficiency of the lysosomal acid alpha-glucosidase (GAA) enzyme described in 1932 by the Dutch pathologist Joannes Cassianus Pompe. The prevalence of PD ranges from 1:40,000 to 1:300,000 births and depends on geographic and ethnic factors. Clinical manifestations may vary from a rapidly progressive disabling disease with cardiomegaly, hepatomegaly, weakness, generalized hypotonia, and death within the first year of life, to a mild presentation characterized by slowly progressive myopathy predominantly involving the skeletal muscles. The laboratory diagnostic gold standard is represented by the determination of the alpha-glucosidase activity. However, the muscle histology may also yield the diagnosis by evaluating the tissular glycogen accumulation. Until recently, supportive measures constituted the unique available therapy. Currently, the administration of the recombinant GAA is being used with promising results. The authors present the case of a 5-month-old boy, previously diagnosed with hypertrophic cardiomyopathy since the age of 2 months, who presented acute heart failure accompanied by biventricular dilation followed by refractory shock and death. The autopsy findings confirmed the glycogen-accumulation disease.

### Keywords

Autopsy; Glycogen Storage Disease Type II; GAA protein, human; Cardiomyopathy; Diagnosis

### CASE REPORT

A 5-month-old boy, previously diagnosed with non-obstructive hypertrophic cardiomyopathy was brought to the emergency facility with a 3-week history of progressive hypoactivity, loss of appetite, decreased diuresis, and vomiting. Fever was absent.

The diagnosis of hypertrophic cardiomyopathy had been made at the age of 2 months. From that time, he began outpatient follow-up at a tertiary hospital, and was prescribed propranolol. An echodopplercardiogram performed 1 month ago showed marked biventricular wall thickening and right

ventricle diastolic dysfunction, but the left ventricle systolic and diastolic functions were preserved.

On the day after admission a new echodopplercardiogram was undertaken, which showed left ventricle hypertrophy and dilation with systolic dysfunction (left ventricular ejection fraction of 38% [Teichholz]), marked mitral insufficiency due to valvular ring dilation, and a non-restrictive pericardial effusion. His clinical status worsened, with pallor, peripheral hypoperfusion (which was non-responsive to volume resuscitation), liver enlargement, and cardiac arrest, which did not respond to routine measures of advanced life support.

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## AUTOPSY FINDINGS

The ectoscopy of a male child corpse weighing 7.3 kg (between the 15th and 50th percentile for the age)<sup>1</sup> and 70 cm of height (97th percentile for the age),<sup>1</sup> was unremarkable.

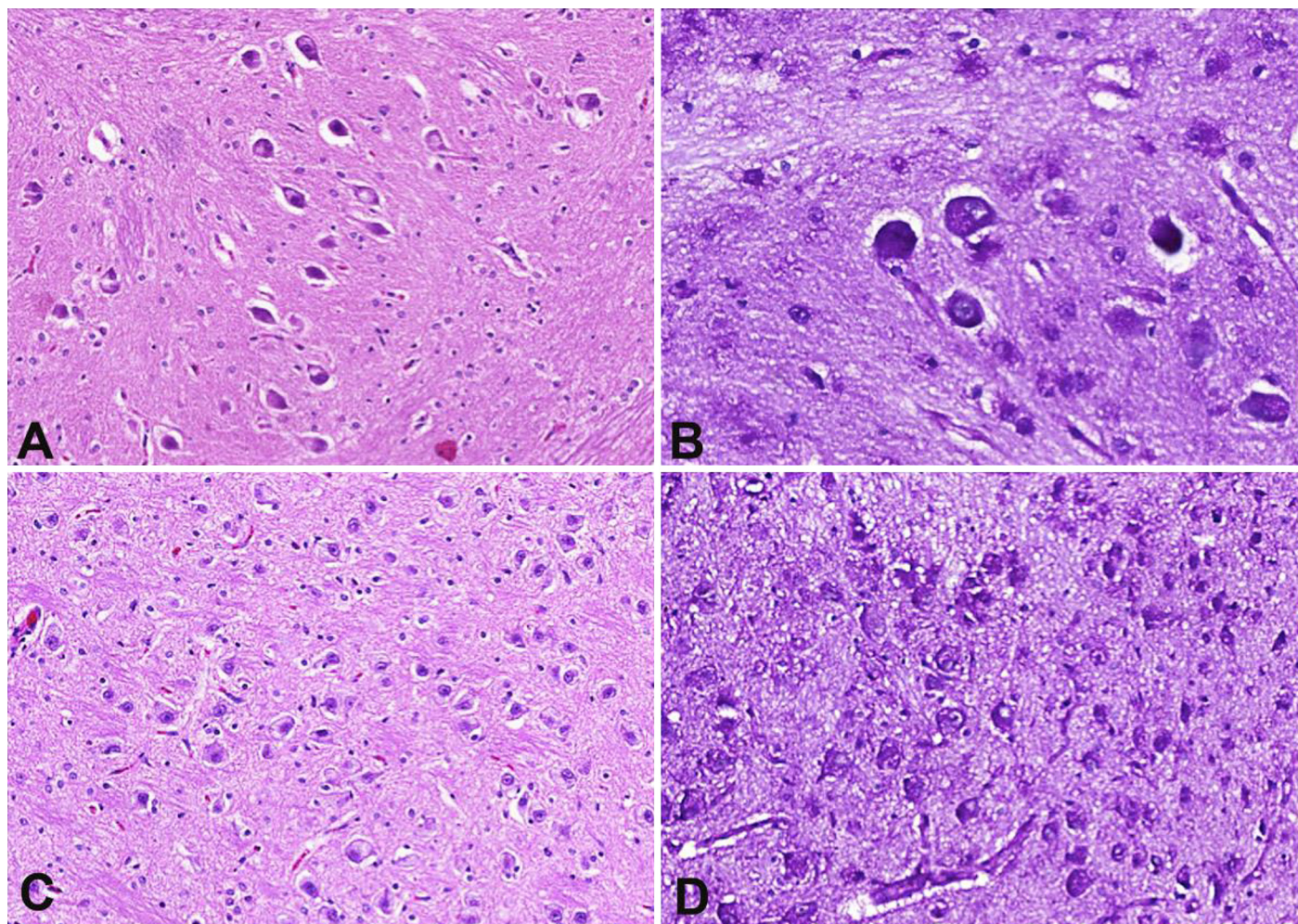
The brain weighed 742 g (reference value [RV]: 413 g) presenting no gross abnormalities. At microscopy, the neurons exhibited periodic acid-Schiff (PAS)-positive material filling vacuoles predominantly in the bulb and pons (Figure 1).

The heart weighed 168 g (over the 95th percentile for the age)<sup>1</sup> with dilated chambers and a thickened interventricular septum that measured 1.2 cm, and a ventricular wall width of 0.7 cm and 1.2 cm in the right and left ventricles, respectively. The microscopic examination revealed a vacuolar PAS-negative myopathy (Figure 2).

The right lung weighed 48 g and the left 78 g (25th percentile for the age)<sup>1</sup> showing lower lobes congestion mainly in the left side. The microscopy evidenced edema, congestion, and alveolar hemorrhage with hemosiderin-loaded macrophages in the alveolar space.

The esophagus was normal, grossly, but presented mild chronic esophagitis with focal ulceration at microscopy.

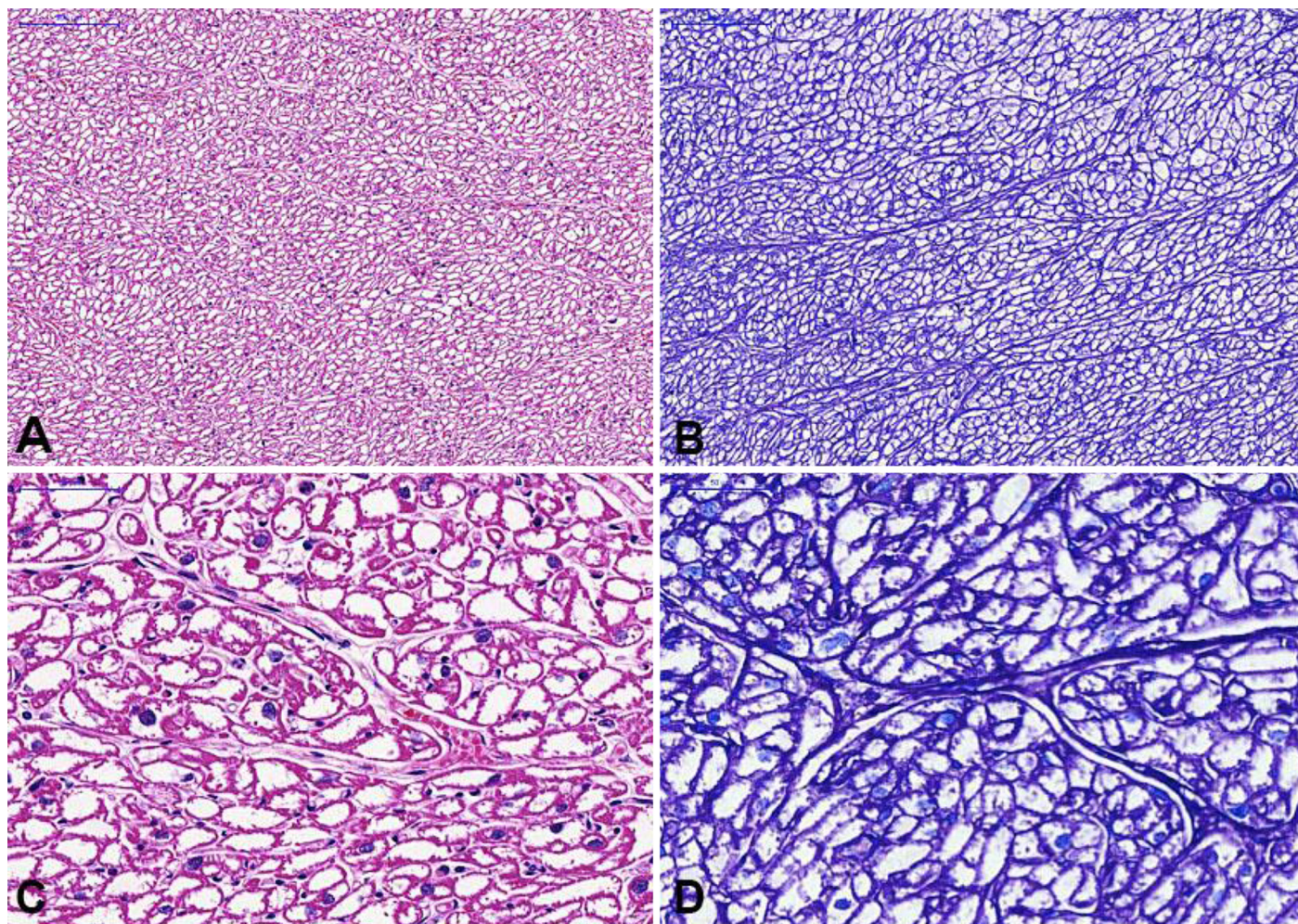
The liver weighed 210 g (between the 5th and 25th percentile for the age),<sup>1</sup> and presented a smooth surface with purplish dots, which were also noticed at the parenchymal cut surface of the subcapsular region. The microscopy evidenced vacuolization of hepatocytes (Figure 3A), filled by PAS-positive material. The right and left kidneys weighed 34 g and 36 g, respectively, (between 75th and 95th percentile for the age)<sup>1</sup> with a nut-brown color and a smooth



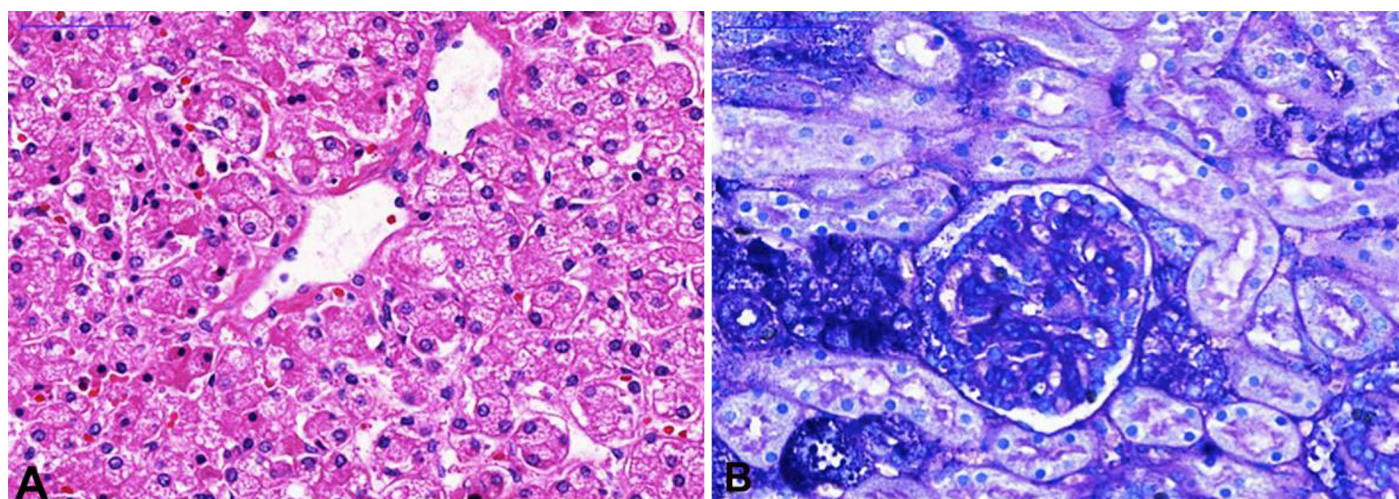
**Figure 1.** Photomicrography of the brain. **A** - Medulla oblongata with vacuoles on neurons (H&E, 200X); **B** - Medulla oblongata with intraneuronal vacuolar neuropathy (PAS, 200X); **C** - Pons showing neurons with vacuolated cytoplasm (H&E, 100X); **D** - Pons with PAS-positive material filling those vacuoles (PAS, 200X).

surface. The microscopic examination showed medullar congestion and thickening of the arterioles, besides the presence of PAS-positive material deposition in the mesangium and Bowman's space (Figure 3B).

The bone marrow was moist and reddish, and showed normal cellularity and preserved hematopoiesis. PAS-positive material was present within the hematopoietic cells (Figure 4A and 4B).



**Figure 2.** Photomicrography of the myocardium. **A** (H&E, 100X) and **B** (PAS, 100X) showing diffuse vacuolar PAS negative myopathy; **C** (H&E, 400X) and **D** (PAS, 400X) myocardiocytes with cytoplasm vacuolization, PAS negative in detail.



**Figure 3.** **A** - Photomicrography of the liver. Diffuse hepatocyte vacuolization (H&E, 400X); **B** - Photomicrography of the kidney. – PAS-positive material deposition in the mesangium and in Bowman's space (PAS, 400X).

The skeletal muscle was grossly normal, but at microscopy, an accumulation of a light material between the fibers was found, as well as vacuolization of the cytoplasm (vacuolar myopathy) with PAS-positive material (Figure 4C and 4D). The same pattern of vacuolar myopathy (PAS positive) was also present in the bladder smooth muscle along with the diaphragm and the tongue.

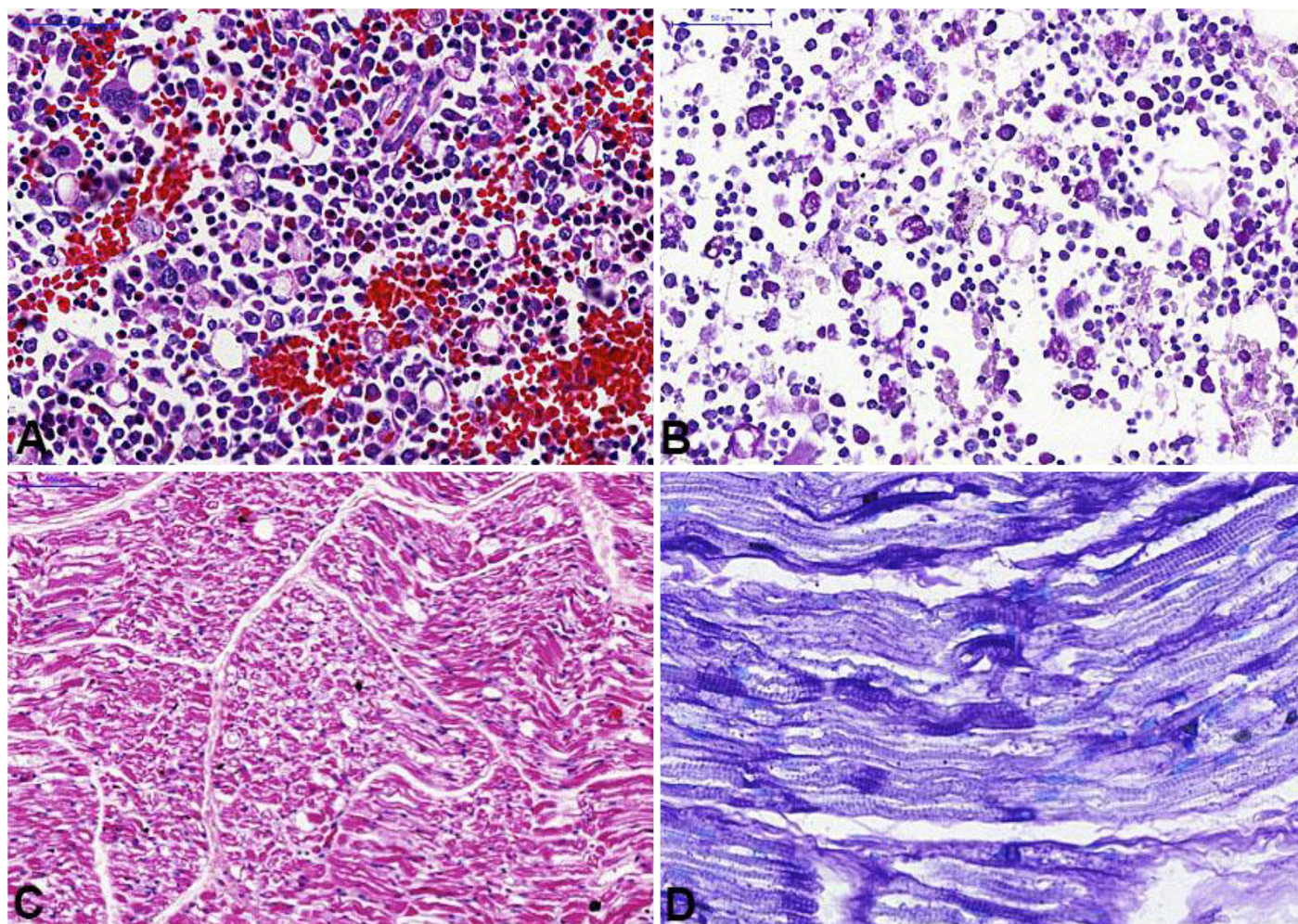
## DISCUSSION

Glycogenosis type II, also known as Pompe disease (PD), or acid maltase deficiency, is a rare autosomal recessive inherited disease caused by the deficiency of the acid alpha-glucosidase (GAA) (also known as lysosomal acid maltase), described by the Dutch pathologist Joannes Cassianus Pompe, in 1932.<sup>2</sup> This enzyme takes part in the degradation process of glycogen into glucose within the muscle fibers.

Mutations in the GAA gene induce varied degrees of enzyme deficiency, resulting in the accumulation of glycogen within the lysosomes.<sup>3</sup> The prevalence of PD ranges between 1:40,000 and 1:300,000 births and is dependent on ethnic and geographical factors.<sup>4</sup>

Phenotypes of GAA deficiency comprise three forms of the disorder; namely the infantile, the childhood, and the late-onset or adult myopathy forms.<sup>5</sup> The infantile form is more frequent among the Afro-descendant and Chinese population, while the adult form is more frequent among the Dutch. The neonatal screening program for lysosomal storage disease showed a higher frequency than expected in Taiwan and Austria.<sup>4,6</sup>

The classic or infantile PD is the more aggressive and severe form, which is characterized by rapidly progressive behavior with marked cardiomegaly, hepatomegaly, weakness, hypotonia, and death due to respiratory failure in the first year of life,<sup>6</sup> as observed



**Figure 4.** **A** and **B** - Photomicrography of the bone marrow. **A** - Intracytoplasmic vacuoles on histiocytes (H&E, 400X); **B** - PAS-positive material inside histiocytes vacuoles (PAS 400X); **C** and **D** - Photomicrography of the skeletal muscle. **C** - Vacuolar myopathy (H&E, 100X); **D** - Skeletal muscle with PAS-positive material deposition (PAS, 400X).

in the patient reported herein. The non-classic or childhood form presents less severe cardiac involvement, the spectrum of which ranges from unaffected to moderate cardiac hypertrophy and cardiac dysfunction.<sup>6</sup> The adult form is characterized by slowly progressive myopathy predominantly involving the skeletal muscles, which starts from the second until the sixth decades of life.<sup>6</sup> The late-onset form may affect respiratory muscles, which will be responsible for significant morbidity.<sup>7</sup> In 2005, Hagemans et al.<sup>8</sup> published a study comprising 54 patients with the late-onset form of the disease showing (with substantial heterogeneity) the mean age of initial symptoms at 28.1 years. However, 85% of the patients had presented mild muscular symptoms during their infancy. In this study, the speed of disease progression was also heterogeneous.

PD physiopathology is based upon the glycogen accumulation secondary to the GAA deficiency, which causes enlargement and rupture of the lysosomes membrane with leakage of its contents into the sarcoplasm directly displacing and disrupting the contractile apparatus. The lysosomal enlargement triggers autophagic vacuoles that act in the cytoplasm and hamper the contractile function of the eventually preserved fibers due to their size, which may reach up to half of the fiber diameter.<sup>9</sup> In this setting, three autophagic mechanisms have been described, namely: (i) microautophagy – when cytoplasmic components are engulfed by the lysosomes; (ii) chaperone-mediated autophagy – when cytosolic hydrosoluble pentapeptide proteins are recognized by molecular chaperones, which transport them into the lysosome through a receptor (LAMP-2A); and (iii) macroautophagy – the most relevant and physiologic mechanism in PD – when proteins or proteic aggregates and organelles are degraded.<sup>7,10</sup> Macroautophagy involves the formation of a double-membrane vesicle that engulfs cytoplasmic constituents and damaged organelles, which fuses with lysosome and degrades its contents. In the context of the lack of intracellular nutrients in PD, macroautophagy provides energy and amino acids.<sup>2,4</sup>

The recombinant GAA is an enzyme that acts in the three autophagic pathways.<sup>4,7,10</sup>

Diagnosis of infantile-onset PD may be suspected in the presence of a massive cardiomegaly on the chest x-ray accompanied by a short PR interval and tall QRS complexes on the electrocardiogram.

The echodopplercardiogram will show hypertrophic cardiomyopathy with or without outflow tract obstruction that becomes dilated and severely dysfunctional in the late stages. Creatinine kinase (CK) determination is altered almost totally in the early forms of the disease, as are aspartate transaminase, alanine transaminase, or lactic dehydrogenase. However, late onset form may present CK within the normal limits.<sup>6</sup>

The current gold-standard diagnostic exam for the confirmation of type II glycogenosis is the determination of GAA activity (markedly reduced) in cultured skin fibroblasts or leukocytes (specifically lymphoid line cells or mononuclear cells). New methods have been developed to determine GAA activity in dried blood spot assay.<sup>2,11</sup> Although sensible, but less specific, the determination of a glucose tetrasaccharide in the urine, the Glc4, can also be used for the diagnosis of PD, but may be altered in other glycogen storage diseases too, requiring clinical correlation for the precise interpretation.<sup>12</sup> The combination of the increased urinary Glc4 determination with the serum activity of GAA achieves approximately 100% of sensitivity for the diagnosis of the infantile form of PD, while normal values for both determinations virtually rule out this diagnosis avoiding further invasive investigation in the form of a muscle biopsy or fibroblast cultures.<sup>6</sup> The use of a non-specific fixative for glycogen preservation can wash-out the glycogen deposits. Alcoholic fixatives are superior on the conservation of such deposits, and the use of these fixatives can demonstrate the glycogen deposits in optical and electron microscopy. However, even when the aqueous based fixatives are used, such as formalin, the presence of vacuolar myopathy can be detected on histological examination

Although the determination of the GAA activity is the first choice examination for the PD diagnostic work-up, molecular analysis of the GAA gene mutations may be useful in suspected cases or in the adult form when residual GAA activity may be present. More than 100 mutations and several variations in the GAA encoding gene were identified and are cataloged in the Erasmus University Medical Center (Rotterdam/Holland). Among the recurrent infantile form, the single base pair deletion  $\Delta 525T$  corresponds to 34% of cases in Holland<sup>13</sup> and 9% in the USA.<sup>14</sup> The exon 18 deletion corresponds to 25% of cases in Holland and Canada, and 5% in the USA.<sup>6,13,14</sup> The leaky IVS1(-13T>G) splice-site mutation accounts

for 50% of the late-onset forms.<sup>14</sup> Some populations exhibit specific mutations, such as the R854X mutation, which is found in many African and African-American cases; the D645E is found among Chinese infantile form cases; the 2741AG->CAGG insertion is found in Turkish cases; and the G925A mutation in European cases.<sup>6</sup>

The muscle biopsy plays an important role in the work-up of PD mainly in the absence of familial history.<sup>2</sup> Although invasive, the histopathology enables a faster diagnosis showing the presence of rounded vacuoles that are irregular in shape and size and filled by PAS-positive material that corresponds to glycogen accumulation within the lysosomes; in advanced cases, this is also dispersed in the cytosol. Lipofuscin accumulation and lipid droplets may also be observed. The vacuoles may also stain intensively to acid phosphatase confirming the vacuoles are secondary lysosomes.<sup>2,15</sup> Ultrastructurally Z-line streaming and thickening, as well as non-specific mitochondrial alterations, may be observed in infantile and juvenile cases.<sup>15</sup> In the adult form, due to the heterogeneity of glycogen accumulation in different muscle groups or different muscle fibers in the same sample, the site of the biopsy may render misdiagnosis. The vacuole frequency is up to 100% in the infantile form, 75% in the childhood form, and 10–50% in the adult form.<sup>15</sup> The amount of glycogen storage may be 10-fold greater in the infantile form when compared with normal muscular fibers; whereas in the late-onset disease this increment is smaller.<sup>6</sup>

The histology of the patient presented herein permitted confirmation of the diagnosis by showing the vacuolization of the cytoplasm with the accumulation of PAS material between the muscle fibers, which is also found in the bladder smooth muscle, the diaphragm, and the tongue. Similar findings, in this case, comprised the involvement of bone marrow, kidneys, and liver.

The PAS-negative vacuolar cardiomyopathy observed in our patient may be a consequence of artifact fixation and wash-out during histological preparation. However, the gross finding of the heart was indeed consistent with PD since the cardiac response to glycogen accumulation resulted in hypertrophy or hypertrophic and dilated cardiomyopathy.

The differential diagnosis of PD comprises other glycogen storage diseases, metabolic myopathy and muscular dystrophy, which may exhibit similarities concerning many of the clinical features as shown in the laboratory work-up.<sup>6,8</sup>

Until recently, specific treatment for PD was not available, and supportive measures were the only reasonable therapy. Limited benefits were achieved with the intake of high protein diets, alanine, and branched-chain amino acids. Enhancing glycogen-breakdown agents like epinephrine and glucagon were tested without benefits.<sup>6</sup> Currently, studies are being undertaken regarding replacement of the lacking enzyme. Clinical trials involving recombinant human GAA replacement in infantile-form patients show increased survival, reduction of the cardiomegaly, and improvement of cardiac and skeletal muscle function. Cardiac function improvement seems to not depend on the precocity of therapy; however, the skeletal muscle response shows better results when the treatment is started before marked muscular damage and even though the response to therapy is less encouraging. The type of muscle fiber, the stage of the disease at the initial therapeutic regimen, the genotype, and the immune response against the recombinant enzyme may play an important roles in the therapeutic success.

Larger clinical trials are being held to evaluate long-term benefits and enzyme replacement dosage. Survival is becoming a reality with such therapy and the outcome of the disease in the treated patients is fundamental.<sup>4,6</sup> Type II glycogenosis genetic therapy is also being developed. Studies show that the expression of the GAA gene in the muscle cells or the secretion of the enzyme by the liver produces a decrement of the disease in rats. Pharmacological chaperones that restore the affected protein morphology and biological function may be a future therapy.<sup>4</sup>

Unfortunately, the patient of this report was not diagnosed early and properly. It is likely that the outcome could have been more promising if the recombinant enzyme replacement, which is currently available, had been used. Besides clinical awareness, all efforts should be focused for an early and precise diagnosis; it is only this way that these patients may experience an extended lifespan.

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**Conflict of interest:** None

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